

09/063, 978

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Amended) A binding assay for sensing analyte mass in a liquid sample, comprising:

- a) immobilizing an array on a surface of a substrate, wherein the array comprises a plurality of microscopic sorbent zones, wherein a microscopic sorbent zone comprises a multi-layer matrix of an analyte binding partner, the matrix extending up to 200 nm vertically from the surface of the substrate;
- b) contacting a defined volume of sample believed to contain an analyte with at least one microscopic sorbent zone, the analyte binding partner in the microscopic sorbent zone being present in excess relative to the analyte, so that any analyte present in the defined volume is substantially depleted from the sample and concentrated on the microscopic sorbent zone to form an analyte capture complex with the analyte binding partner;
- c) tagging the analyte capture complex with a fluorescent label;
- d) illuminating the microscopic sorbent zone with a laser in the absence of liquid; and
- e) detecting fluorescence emissions from any microscopic sorbent zone having an analyte capture complex tagged with a fluorescent label, thereby determining the analyte mass harvested from the defined volume of sample.

2. (Previously Presented) An assay according to claim 27 wherein the substrate is selected from the group consisting of polycarbonate, polystyrene, polyethylene, polypropylene, and polymethylmethacrylate.

3. (Original) An assay according to claim 1 wherein the substrate is a film, sheet, strip, particle, or microtiter plate.
4. (Original) An assay according to claim 1 wherein the analyte binding partner is immobilized by covalent binding to the substrate.
5. (Original) An assay according to claim 1, the sorbent zones further comprising a first binding partner attached to the substrate, the first binding partner forming a first binding complex with a conjugate, the conjugate comprising a first ligand and the analyte binding partner, wherein the first ligand binds specifically with the first binding partner and the analyte binding partner can bind specifically with the analyte.
6. (Original) An assay according to claim 5 wherein the first binding partner is avidin or streptavidin and the first ligand is biotin.
7. (Original) An assay according to claim 6 wherein the conjugate is biotinylated antibody.
8. (Original) An assay according to claim 5 wherein the first binding partner is immobilized by covalent attachment to the substrate.
9. (Original) An assay according to claim 1 wherein the tagging step further comprises: incubating the analyte capture complex with a labeled binding partner, the labeled binding partner having a fluorescent label and being capable of binding to the analyte capture complex.
10. (Original) An assay according to claim 9 wherein the labeled binding partner comprises an antibody.
11. (Original) An assay according to claim 1 wherein the fluorescent label is a cyanine dye.

12. (Original) An assay according to claim 11 wherein the cyanine dye is selected from the group consisting of La Jolla Blue, Cy5, BCy5, DBCy5, Cy7, BCy7, and DBCy7.

13. (Original) An assay according to claim 1 wherein the defined volume of sample is from about 20 μ l to about 500 μ l.

14. (Original) An assay according to claim 1 wherein the amount of the analyte binding partner immobilized in a sorbent zone is from 10^5 to about 10^{12} molecules of analyte binding partner.

15. (Original) An assay according to claim 1 wherein about 10^5 to about 10^{10} molecules of analyte are detected per sorbent zone.

16. (Original) An assay according to claim 1 wherein the diameter of the sorbent zones is about 60 μ m to about 500 μ m.

17. (Original) An assay according to claim 1 wherein the analyte binding partner is an antigen, antibody, oligonucleotide, or receptor.

18. (Original) An assay according to claim 1, wherein the array of sorbent zones comprises a plurality of different analyte binding partners.

19. (Original) An assay according to claim 18, the sorbent zones further comprising at least two subsets, wherein a first subset of sorbent zones contains a first analyte binding partner and a second subset of sorbent zones contains a second analyte partner.

20. (Original) An assay according to claim 1 wherein the immobilizing step further comprises dispensing droplets using a printer jet to form the array of sorbent zones.

21. (Original) An assay according to claim 1 wherein the volume of the droplets is about 80 pl to about 1 nl.

22. (Original) An assay according to claim 1 wherein the illuminating step is conducted by directing near infrared emissions through a prism coupler, into the substrate, thereby producing evanescent wave excitation of fluorescence.

23. (Amended) An analyte binding array for harvesting analyte from a liquid sample, the array comprising a plurality of microscopic sorbent zones immobilized on a surface of a substrate, wherein a microscopic sorbent zone comprises a multi-layer matrix of an analyte binding partner, the matrix extending up to 200 nm vertically from the surface of the substrate, the analyte binding partner being present in an amount sufficient to substantially deplete the analyte from a sample and concentrate the analyte on the microscopic sorbent zone, the microscopic zone being from about 60 to about 500 μm in diameter and the sample containing about 10^5 to about 10^{10} molecules of analyte per 100 μl of the sample, wherein a volume of the sample is from 20 to 500 μl .

24. (Original) An array according to claim 23, wherein the array of sorbent zones comprises a plurality of different analyte binding partners.

25. (Original) An array according to claim 24, the sorbent zones further comprising at least two subsets, wherein a first subset of sorbent zones contain a first analyte binding partner and a second subset of sorbent zones contain a second analyte partner.

26. (Amended) A kit for use in a binding assay that senses analyte mass in a liquid sample of a defined volume, comprising an analyte binding array and a container comprising labeled binding partner,

wherein the analyte binding array comprises a plurality of microscopic sorbent zones immobilized on a surface of a substrate, wherein a microscopic sorbent zone comprises a multi-layer matrix of an analyte binding partner, the matrix extending up to 200 nm vertically from the surface of the substrate, the analyte binding partner being present in excess relative to the analyte, so that any analyte present in the defined volume of the sample is substantially depleted from the sample and concentrated on the microscopic sorbent zone to form an analyte capture complex with the analyte binding partner, and

the labeled binding partner having a fluorescent label and being capable of binding to an analyte bound by an analyte binding partner.

27. (Previously Presented) An assay of claim 1, wherein the substrate is made of an insoluble non-porous material.

28. (Previously Presented) An assay of claim 1, wherein a binding capacity of the microscopic sorbent zone of $150 \mu\text{m}$ in diameter is about 10^{10} analyte molecules.

29. (Previously Presented) The assay of claim 4, wherein the immobilizing step a) further comprises:

- a1) derivatizing the binding partner with a photolabile linker moiety to obtain a derivatized binding partner;
- a2) drying the derivatized binding partner on the substrate; and
- a3) exposing the substrate to UV radiation.

30. (Previously Presented) The assay of claim 14, wherein the amount of the analyte binding partner immobilized in the sorbent zone with a diameter from 60 μm to 500 μm is from 10^9 to 10^{12} molecules.

31. (Previously Presented) The analyte binding array of claim 23, wherein the amount of the analyte binding partner immobilized in the sorbent zone is from 10^9 to 10^{12} molecules.

32. (Previously Presented) The kit of claim 26, wherein the amount of the analyte binding partner immobilized in the sorbent zone with a diameter from 60 μm to 500 μm is from 10^9 to 10^{12} molecules.

33. (Amended) A binding assay for sensing analyte mass in a liquid sample, comprising:

a) immobilizing an array on a surface of a substrate, wherein the array comprises a plurality of microscopic sorbent zones, wherein each microscopic sorbent zone comprises a multi-layer matrix of an analyte binding partner, the matrix extending up to 200 nm vertically from the surface of the substrate, wherein the amount of the analyte binding partner immobilized in the sorbent zone with a diameter from 60 μm to 500 μm is from 10^9 to 10^{12} molecules;

b) contacting a defined volume of sample believed to contain an analyte with at least one microscopic sorbent zone, whereby analyte present in the defined volume is substantially depleted from the sample and concentrated on the microscopic sorbent zone to form an analyte capture complex with the analyte binding partner;

c) tagging the analyte capture complex with a fluorescent label; and

d) detecting fluorescence emissions from the microscopic sorbent zone to determine the analyte mass harvested from the defined volume of sample.

34. (Previously presented) A binding assay for sensing analyte mass in a liquid sample, comprising:

- a) derivatizing a binding partner with a photolabile linker moiety to obtain a derivatized binding partner;
- b) applying aliquots of the derivatized binding partner to a substrate;
- c) exposing the substrate to UV radiation to immobilize the analyte binding partner, whereby an array of microscopic sorbent zones comprising the analyte binding partner forms;
- d) contacting a defined volume of sample believed to contain an analyte with at least one microscopic sorbent zone, the analyte binding partner in the microscopic sorbent zone being present in excess relative to the analyte, so that any analyte present in the defined volume is substantially depleted from the sample and concentrated on the microscopic sorbent zone to form an analyte capture complex with the analyte binding partner;
- e) tagging the analyte capture complex with a fluorescent label;
- f) illuminating the microscopic sorbent zone with a laser in the absence of liquid; and
- g) detecting fluorescence emissions from any microscopic sorbent zone having an analyte capture complex tagged with a fluorescent label, thereby determining the analyte mass harvested from the defined volume of sample.

35. (Amended) An analyte binding array for harvesting analyte from a liquid sample, the array comprising a plurality of microscopic sorbent zones immobilized on a surface of a substrate, wherein a microscopic sorbent zone comprises an analyte binding partner, the analyte binding partner being present in an amount from 10^9 to 10^{12} molecules per each sorbent zone with a diameter from 60 μm to 500 μm .

36. (Amended) The analyte binding array of claim 35, wherein the analyte binding partner forms a multi-layer matrix in the sorbent zone, the matrix extending up to 200 nm vertically from the surface of the substrate.

37. (New) The binding assay of claim 1 or 33, wherein the binding partner is immobilized on the surface of the substrate by covalent immobilization.

38. (New) The binding assay of claim 1 or 33, wherein the binding partner is immobilized on the surface of the substrate by non-covalent immobilization.

39. (New) The analyte binding array of claim 23 or 35, wherein the binding partner is immobilized on the surface of the substrate by covalent immobilization.

40. (New) The analyte binding array of claim 23 or 35, wherein the binding partner is immobilized on the surface of the substrate by non-covalent immobilization.

41. (New) The kit of claim 26, wherein the binding partner is immobilized on the surface of the substrate by covalent immobilization.

42. (New) The kit of claim 26, wherein the binding partner is immobilized on the surface of the substrate by non-covalent immobilization.